# MODE OF ACTION OF PIPERONYL BUTOXIDE AS A SYNERGIST FOR PYRETHRUM

BY

NORMAN WILLISTON EARLE

B.S., University of Illinois, 1949
M.S., University of Illinois, 1950

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1952

URBANA, ILLINOIS

1952

# MODE OF ACTION OF PIPERONYL BUTOXIDE AS A SYNERGIST FOR PYRETHRUM

BY

NORMAN WILLISTON EARLE

B.S., University of Illinois, 1949
M.S., University of Illinois, 1950

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1952

URBANA, ILLINOIS

1952

## ACKNOWLEDGEMENTS

These investigations were carried out under the able guidance of Dr. C. W. Kearns of the Department of Entomology to whom the author wishes to express a sincere appreciation for his invaluable assistance and opportune counsel. The project was supported by a research grant from Gulf Research and Development Company. The members of the entomology and chemistry sections of this company also did much to encourage the work and gave freely of suggestions and essential materials. Special thanks are due Mr. A. C. Miller who called attention to many problems which were in need of solution and who, together with Dr. John Pellegrini and Mr. Ridge Sharpless, contributed considerable time and effort to the preparation of a sample of radioactive pyrethrum which has greatly facilitated the present studies. The author also wishes to express his gratitude to Dr. Eddie Vinson for proofreading a portion of this thesis and to Mr. Herbert Moorefield who carefully prepared the plates in the appendix.

R. UNIV. BIBLIOTHEEK LEIDEN

## MODE OF ACTION OF PIPERONYL BUTOXIDE AS A SYNERGIST FOR PYRETHRUM

Early investigators of the mode of action of synergists were primarily concerned with the study of the effects on irritable tissues as determined by histological techniques. Their conclusions, that synergists inhibit the recovery of pyrethrum-treated insects by damaging certain portions of the nervous system, have often been questioned but not until recently have any attempts been made to offer supporting evidence for alternate hypotheses. Chamberlain (1950) first presented data which contained evidence supporting the hypothesis which requires that enzymes, most likely esterases and lipases, capable of catalyzing the degradation of pyrethrins, are partially inhibited by synergists. In reviewing the literature most of the data can be reconciled with this hypothesis (Lindquist *et al* 1947), Wilson (1949) ). Most of the experiments described in this thesis were designed to test this hypothesis.

The report that piperonyl butoxide is at least capable of partially inhibiting the enzymatic hydrolysis of pyrethrins *in vitro* was confirmed. Although hydrolysis is considered the most likely mechanism of pyrethrins detoxication, there is reason to suspect that there are other mechanisms; some of the tests with $C^{14}$ labeled pyrethrins raise this question.

That piperonyl butoxide does reduce the rate of detoxication of pyrethrins *in vivo* has been established in several ways using both biological and radioactive analysis procedures. It was shown by assaying biologically the extracts of treated *Sarcophaga* with mosquito larvae that piperonyl butoxide reduces the rate of degradation of pyrethrins. In a somewhat similar test, using the blood of treated American roaches rather than extracts of the entire insects, it was shown that the rate of decline in toxicity of the blood, as measured by its effect on *Sarcophaga*, was reduced by synergist treatment.

The period of temporary paralysis of *Sarcophaga* induced by the injection of small amounts of pyrethrins served as a criterion for the detection of small amounts of pyrethrins. By injecting two or more dosages of pyrethrins, and by calculation the average values for the resulting periods of paralysis, a detoxication rate, expressed as γg. of pyrethrins detoxified per fly per unit time, could be obtained. Applying this procedure to the study of the effect of synergist treatment, it was found that sarcophagid flies, which had been treated with piperonyl butoxide, could no longer detoxify pyrethrins as rapidly as the untreated flies.

Petroleum ether extracts of flies treated with a combination of $C^{14}$ pyrethrins and piperonyl butoxide were significantly more radioactive than the corresponding extracts of flies treated with labeled pyrethrins only. To confirm the belief that the activities of the petroleum ether extracts were proportional to the amounts of undegraded pyrethrins present, extracts prepared from flies treated with non-radioactive pyrethrins, with and without piperonyl butoxide, were assayed biologically using *Sarcophaga* and were shown to be toxic. As was expected, the extract of the synergist treated houseflies was most toxic, indicating that piperonyl butoxide was responsible for a partial inhibition of pyrethrins detoxication.

The relatively poor efficiency of nearly all pyrethrum synergists when administered in combination with the synthetic homologue, allethrin, still cannot be explained satisfactorily. It was demonstrated that allethrin is more resistant to hydrolysis *in vitro* and less susceptible to detoxication *in vivo* than pyrethrins. This relative stability of allethrin may explain the low degree of activation in the presence of synergists since the protection from detoxication would not be as noticeable in the case of a stable compound as it would in the case of a less stable one such as pyrethrum. On the other hand, some of the data suggest that the failure of piperonyl butoxide to act as an effective allethrin synergist may simply be due to the fact that the synergist does not inhibit those enzyme systems which are responsible for the degradation of allethrin.

The mechanism of pyrethrins resistance of a strain of houseflies developed under laboratory conditions was studied with the aid of $C^{14}$ labeled pyrethrins. It was concluded that the resistance was due primarily to a relatively impermeable cuticle; a low sensitivity to pyrethrins at the site of action may also be a contributing factor.

An attempt was made to discover the reason for the relatively low susceptibility of newly emerged flies. The data indicated that some factor other than a more efficient detoxication mechanism was responsible, since contrary to expectations, old flies *(Sarcophaga)* were able to detoxify pyrethrins more rapidly than younger flies.

## BIBLIOGRAPHY

Chamberlain, Roy W. 1950. An investigation on the action of piperonyl butoxide with pyrethrum. Amer. Journ. Hygiene, 52(2): 153-183.

Lindquist, A. W., A. H. Madden, and H. G. Wilson. 1947. Pretreating houseflies with synergists before applying pyrethrin sprays. J. Econ. Ent., 40:426-427.

Wilson, C. A. 1949. Piperonyl butoxide, piperonyl cyclonene, and pyrethrum applied to selected parts of individual flies. J. Econ. Ent. 42:423-428.

## VITA

Norman Williston Earle was born September 7, 1927 in San Juan, Puerto Rico. He completed his grammer school education at Doctor Howard School in Champaign in 1940 and his high school education at Champaign High School in June 1945. At this time he entered the University of Illinois. In February 1946 his education was interrupted when he was called to active duty in the Armed Services. After serving in the European Theater of Operations as a radio operator he was discharged in April 1947. In the fall of that same year he re-entered the University of Illinois and received a Bachelor of Science degree in chemistry in February 1949. During the summer of 1949 he was employed by the Julius Hyman Company as a chemist in their research laboratory. In September 1949 he resumed his graduate studies in entomology at the University of Illinois, receiving a Master of Science degree in June 1950. He was granted an industrial fellowship during the school years 1950-1952.

He belongs to the American Association of Economic Entomologists and the American Chemical Society.